CLAIMS

1. A method of comparing copy numbers of different DNA sequences in a subject cell or cell population comprising the steps of:

a) extracting the DNA from the subject cell or from a number of cells of the subject cell population;

b) amplifying said extracted subject DNA, if necessary;

c) labeling the subject DNA;

d) hybridizing said labeled subject DNA in situ to reference metaphase chromosomes after substantially removing from the labeled DNA those repetitive sequences that could bind to multiple loci in the reference metaphase chromosomes, and/or after blocking the binding sites for those repetitive sequences in the reference metaphase chromosomes by prehybridization with appropriate blocking nucleic acids, and/or blocking those repetitive sequences in the labeled DNA by prehybridization with appropriate blocking nucleic acid sequences, and/or including such blocking nucleic acid sequences for said repetitive sequences during said hybridization, wherein the DNA sequences in the labeled subject DNA that bind to single copy sequences in the reference metaphase chromosomes are substantially retained, and those single copy DNA sequences as well as their binding sites in the reference metaphase chromosomes remain substantially unblocked both before and during the hybridization;

e) rendering the bound, labeled DNA sequences visualizable, if necessary;

f) observing and/or measuring the intensity of the signal from the labeled subject DNA sequences as a function of position on the reference metaphase chromosomes; and

g) comparing the copy numbers of different DNA sequences of the subject DNA by comparing the signal intensities at different positions on the reference metaphase chromosomes, wherein the greater the signal intensity at a given position, the greater the copy number of the sequences in the subject DNA that bind at that position.

2. A method according to Claim 1 wherein the repetitive sequences that could bind to multiple loci in the reference metaphase chromosomes are high copy number repetitive sequences.

3. A method according to Claim 1 wherein said subject cell or cell population is derived from a clinical specimen.

4. A method according to Claim 1 wherein the copy number of a subject DNA sequence binding at one position in the reference metaphase chromosomes relative to the copy number of a sequence binding at another position is quantified by measuring the ratio of the signal intensities at the two locations.

5. A method according to Claim I further comprising the addition of an unlabeled nucleic acid to said hybridization mixture wherein said unlabeled nucleic acid has a sufficient number of nucleic acid sequences substantially complementary to the sequences in the reference metaphase chromosomes to prevent saturation of the binding sites in the reference metaphase chromosomes by the labeled subject DNA.

6. A method according to Claim 1 wherein the reference metaphase chromosomes are human and prehybridized with human genomic DNA and/or human genomic DNA enriched in high copy repetitive sequences, and wherein human genomic DNA and/or human genomic DNA enriched in high copy repetitive sequences are included in the hybridization.

7. A method according to Claim 1 wherein the labeled subject DNA is tumor DNA.

8. A method according to Claim 1 wherein the labeled subject DNA is fetal DNA.

9. A method according to Claim 1 wherein in step (f) and/or (g), an image analysis system is used.

10. A method according to Claim 1 wherein said labeled subject DNA sequences are labeled with a label selected from the group consisting of fluorochromes, ligands, radionuclides, chemiluminescers, enzyme substrates, enzyme co-factors, particles and dyes.

11. A method according to Claim 10 wherein the label is a fluorochrome.

12. A method according to Claim 1 wherein the amplifying of step (b) comprises a polymerase chain reaction (PCR) procedure.

13. A method according to Claim 1 wherein the amplifying of step (b) is a non-polymerase chain reaction (non-PCR) procedure.

14. A method according to Claim 1 wherein signal intensities significantly greater than average indicate the presence and locations on the reference metaphase chromosomes of sequences that are duplicated or amplified in the subject cell or cell population whereas signal intensities that are significantly less than average indicate the presence and locations of sequences that are deleted.

15. A method according to Claim 1 wherein the subject DNA is extracted from formalin-fixed and/or paraffin-embedded archived tissue specimens.

16. A method according to Claim 14 wherein said amplifications contain(s) sequences from one or more oncogenes, and wherein said deletions include(s) sequences from one or more tumor suppressor genes.

17. A method according to Claim 1 wherein the reference metaphase chromosomes are from an antenna cell line.

18. A method according to Claim 1 wherein an ensemble of amplifications, duplications and/or deletions is detected simultaneously in a tumor cell or cells, and from the ensemble, an association to the tumor's probable behavior is made.

19. A method of comparing copy numbers of different RNA sequences in a subject cell or cell population comprising the steps of:

a) extracting the RNA from the subject cell or from a number of cells of the subject cell population;

b) amplifying said extracted subject RNA, if necessary;

c) labeling the subject RNA;

d) hybridizing said labeled subject RNA in situ to reference metaphase chromosomes after substantially removing from the labeled RNA those repetitive sequences that could bind to multiple loci in the reference metaphase chromosomes, and/or after blocking the binding sites for those repetitive sequences in the reference metaphase chromosomes by prehybridization with appropriate blocking nucleic acids, and/or blocking those repetitive sequences in the labeled RNA by prehybridization with appropriate blocking nucleic acid sequences, and/or including such blocking nucleic acid sequences for said repetitive sequences during said hybridization, wherein the RNA sequences in the labeled subject RNA that bind to single copy sequences in the reference metaphase chromosomes are substantially retained, and those RNA sequences as well as their binding sites in the reference metaphase chromosomes remain substantially unblocked both before and during the hybridization;

e) rendering the bound, labeled RNA sequences visualizable, if necessary;

f) observing and/or measuring the intensities of the signals from the labeled subject RNA sequences as a function of position on the reference metaphase chromosomes; and

g) comparing the copy numbers of different RNA sequences of the subject RNA by comparing the signal intensities at different positions on the reference metaphase

chromosomes, wherein the greater the signal intensity at a given position, the greater the copy number of the sequences in the subject RNA that bind at that position.

20. A method of comparing copy numbers of different DNA sequences in one subject cell or cell population relative to copy numbers of substantially identical sequences in another cell or cell population, said method comprising the steps of:

a) extracting the DNA from both of the subject cells or cell populations;

b) amplifying said extracted subject DNAS, if necessary;

c) differentially labeling the subject DNAS;

d) hybridizing said differentially labeled subject DNAs in situ to reference metaphase chromosomes after substantially removing from the labeled DNAs those repetitive sequences that could bind to multiple loci in the reference metaphase chromosomes, and/or after blocking the binding sites for those repetitive sequences in the reference metaphase chromosomes by prehybridization with appropriate blocking nucleic acids, and/or blocking those repetitive sequences in the labeled DNA by prehybridization with appropriate blocking nucleic acid sequences, and/or including such blocking nucleic acid sequences for said repetitive sequences during said hybridization;

e) rendering the bound, differentially labeled DNA sequences visualizable, if necessary;

f) observing and/or measuring the intensities of the signals from each subject DNA, and the relative intensities, as a function of position along the reference metaphase chromosomes; and

g) comparing the relative intensities among different locations along the reference metaphase chromosomes wherein the greater the intensity of the signal at a

location due to one subject DNA relative to the intensity of the signal due to the other subject DNA at that location, the greater the copy number of the sequence that binds at that location in the first subject cell or cell population relative to the copy number of the substantially identical sequence in the second subject cell or cell population that binds at that location.

21. A method of quantitatively comparing copy numbers of different DNA sequences in one subject cell or cell population relative to copy numbers of substantially identical sequences in another subject cell or cell population, said method comprising steps (a) through (e) of Claim 20 and steps of:

f. measuring the intensities of the signals from each of the bound subject DNAs and calculating the ratio of the intensities as a function of position along the reference metaphase chromosomes to form a ratio profile; and

g. quantitatively comparing the ratio profile among different locations along the reference metaphase chromosomes, said ratio profile at each location being proportional to the ratio of the copy number of the DNA sequence that binds to that location in the first subject cell or cell population to the copy number of a substantially identical sequence in the second cell or cell population.

22. A method according to Claim 20 further comprising comparing copy numbers of different DNA sequences in more than two subject DNAs wherein the comparing is done pairwise between the signals from each subject DNA.

23. A method to determine the ratio of copy numbers of different DNA sequences in one subject cell or cell population to copy numbers of substantially identical sequences in another cell or cell population, said method comprising the steps of (a) through (f) of Claim 21 and steps of:

g. determining the average copy number of a calibration sequence in both subject cells or cell populations, said calibration sequence being substantially identical to a single copy sequence in the reference metaphase cells; and

h. normalizing the ratio profile calculated in (f) so that at the calibration position, the ratio profile is equal to the ratio of the average copy numbers determined in (g), the normalized ratio profile at any other location along the reference metaphase chromosomes thereby giving the ratio of the copy numbers of the DNA sequences in the two subject DNAs that would bind at that location.

24. The method according to Claim 23 further comprising determining the ratio of copy numbers of DNA sequences in more than two subject DNAs wherein the comparing is done pairwise between signals from each subject DNA.

25. The method according to Claim 23 further comprising determining the copy numbers for more than one calibration position in step (g); and doing the normalizing in step (h) to obtain the best fit of the ratio profile to the calibration positions.

26. The method according to Claim 24 wherein more than one calibration position is used in step (g).

27. A method for comparing copy numbers of different DNA sequences in a test cell or cell population, said method comprising applying steps (a) through (f) of Claim 20 wherein one of the subject cells or cell populations is the test cell or cell population and the other is a normal cell or cell population, and step (g) of comparing the relative intensities among different locations along the reference metaphase chromosomes, wherein the greater the relative intensity at a location, the greater the copy number of the sequence in the test cell or cell population that binds to that location, except for sex chromosomes where the comparison needs to take into account the known differences in copy numbers of sequences in the sex chromosome in relation to those on the autosomes in the normal subject cell or cell population.

28. A method for comparing the copy number of different DNA sequences in a test cell or cell population, said method comprising applying steps (a) through (f) of claim 21 wherein one of the subject cells or cell populations is the test cell or cell population, and the other is a standard cell or cell population wherein the known copy numbers of the DNA sequences that bind to different positions on the reference metaphase chromosomes is known and steps:

g. adjusting the ratio profile at each location along the reference metaphase chromosomes by multiplying the ratio profile by the known copy number of DNA sequences in the standard cell or cell population that bind there; and h. comparing the adjusted ratio profiles at different locations along the reference metaphase chromosomes wherein the greater the adjusted ratio profile at a location, the greater the copy number of the DNA sequence in the test cell or cell population that binds there.

29. A method of determining the ratios of the copy numbers of different DNA sequences in a test cell or cell population, said method comprising applying steps (a) through (g) of Claim 28 and calculating the ratio of the copy number of a DNA sequence in the test cell or cell population that binds to one location on the reference metaphase chromosomes to the copy number of a sequence that binds to another location by dividing the adjusted ratio profile at the location of the first sequence by that at the location of the second.

30. The method of Claim 28 where the standard cell or cell population is normal.

31. A method for determining the copy number of different DNA sequences in a test cell or cell population, said method comprising

applying steps (a) through (g) of claim 28;

h. determining the copy number of a calibration sequence in the test cell or cell population that is substantially identical to a single copy sequence in the reference cells; and

i. normalizing the adjusted ratio profile so that at the location of the calibration sequence on the reference metaphase chromosomes, the normalized, adjusted ratio profile is equal to the copy number of the calibration sequence determined in (h), the value of the normalized, adjusted ratio profile at another location then being equal to the copy number of the DNA sequence in the test cell or cell population that binds at that location.

32. The method of Claim 31 wherein two or more calibration sequences are used, and the adjusted ratio profile is normalized to get the best fit to the copy numbers of the ensemble of calibration sequences.

33. The method of Claim 31 wherein determination of the copy number of the calibration sequence is by in situ hybridization.

34. The method of Claim 29 wherein the standard cell(s) is normal.

35. The method of Claim 20 wherein the subject and reference genomes are human.

36. The method of Claim 31 wherein the standard cell(s) is normal.

37. The method according to Claim 1 wherein said reference metaphase chromosomes are normal.

38. The method according to Claim 20 wherein said reference metaphase chromosomes are normal.

39. The method according to Claim 23 wherein the copy number determinations of step (g) are done by in situ hybridization.

40. A method of comparing copy numbers of different RNA sequences in one subject cell or cell population relative to copy numbers of substantially identical sequences in another cell or cell population, said method comprising the steps of:

a) extracting the RNA from both of the subject cells or cell populations;

b) amplifying said extracted subject RNAS, if necessary;

c) differentially labeling the subject RNAS;

d) hybridizing said differentially labeled subject RNAs in situ to reference metaphase chromosomes after substantially removing from the labeled RNAs those repetitive sequences that could bind to multiple loci in the reference metaphase chromosomes, and/or after blocking the binding sites for those repetitive sequences in the reference metaphase chromosomes by prehybridization with appropriate blocking nucleic acids, and/or blocking those repetitive sequences in the labeled RNA by prehybridization with appropriate blocking nucleic acid sequences, and/or including such blocking nucleic acid sequences for said repetitive sequences during said hybridization;

e) rendering the bound, differentially labeled RNA sequences visualizable, if necessary;

f) observing and/or measuring the intensities of the signals from each subject RNA, and the relative intensities as a function of position along the reference metaphase chromosomes; and

g) comparing the relative intensities among different locations along the reference metaphase chromosomes wherein the greater the intensity of the signal at a location due to one subject RNA relative to the intensity of the signal due to the other subject RNA at that location, the greater the copy number of the sequence that binds at

that location in the first subject cell a cell population relative to the copy number of the substantially identical sequence in the second subject cell or cell population.

41. A method of detecting amplification of a certain sequence or group of sequences in a subject cell or cell population, comprising steps (a) through (e) of Claim 1 wherein the in situ hybridization is targeted to antenna cells in which the DNA sequences to be tested for is or are amplified, and examining the reference cell for regions that are hybridized significantly more intensely than others, the presence of such regions indicating amplifications of the sequences which are being tested.

42. The method of Claim 41 wherein the hybridization is to interphase antenna cells.

43. The method of Claim 41 wherein the hybridization is to metaphase chromosomes from the antenna cells.

44. The method according to Claim 20 wherein said differentially labeled subject DNAs are hybridized in situ simultaneously.